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THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant :	Charles D. Jones	
Serial No.:	331,042	Group Art Unit: 121
Filed :	December 16, 1981	Examiner: R. Schwartz
For :	ANTIESTROGENIC AND ANTI-ANDROGENIC BENZOTHIOPHENE	
Docket No.:	X-5526A	

DECLARATION UNDER 37 C.F.R. 1.132

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Commissioner of Patents and Trademarks

Washington, D. C. 20231

Sir:

Larry J. Black declares as follows:

Since about 1965, I have been employed in the central nervous system-endocrinology research division of Eli Lilly and Company. I obtained my Bachelor of Science degree in Biological Sciences from Indiana Central College in 1966. In or about 1968, I was made responsible for studies on the mechanism of action of estrogens and antiestrogens, and of progestational and anti-fertility agents. I supervise the testing of such drugs and assure the validity of the test systems. I am named as inventor on two U.S. patents, and am an author of about ten scientific papers.

I have directly supervised the testing of a number of compounds related to the above-named patent application. This paper reports the results of a number of such tests. The purpose of these reports is to compare results obtained from testing 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-(2-piperidinoethoxy)benzoyl]-benzo[b]thiophene (compound I) with the results from 6-hydroxy-2-(4-hydroxyphenyl)-3-[4-(2-pyrrolidinoethoxy)benzoyl]benzo[b]-thiophene (compound II). Some salts of the compounds are also tested.

In some cases, the two compounds were tested side by side in the same test. In other cases, the tests using the two compounds were run at different times. It is my experience with the test methods concerned that results run at different times may be compared, because the test methods are reasonably reproducible. The identifying numbers of the tests are recited in the data tables below, so that the reader can tell the origin of each line of data and can, if he wishes, find all of the data obtained from a given test. Untreated controls are provided in all tests and in all data tables so that base line conditions are clearly known.

Some tests of pertinent compounds are not presented, because there is no corresponding compound for comparison. For example, the compound of the above-named patent application wherein R and R^1 are both methyl has been tested, but is disregarded because the corresponding ether of compound II has not been tested. Similarly, the citrate of compound II is disregarded because the citrate of compound I has not been prepared.

A great many tests of both compound I and compound II have been carried out. Compound II was discovered first, and a very large amount of testing was carried out on it because it appeared to be an antiestrogen of an entirely different and superior type, compared with previously known antiestrogenic compounds. Compound I was synthesized later, and is of the same type as compound II and markedly superior to it. Thus, the existence of the new type of antiestrogens had been confirmed in the testing of compound II, and it was only necessary to test compound I sufficiently to prove that it is the compound of choice of the new type.

No clinical comparisons of compounds I and II are available. Compound II has been very extensively studied, because it

was the first of its class to be discovered, and its unusual properties have been published. For example, it is the subject of a chapter by myself in Hormone Antagonists, M.K. Agarwal, editor, Walter D. Gruiter, Berlin, New York (1982). A reprint of the chapter is enclosed. However, before compound II reached a clinical stage, it was displaced by the later synthesized compound I, which is distinctly more advantageous even than is compound II. Thus, compound II will not be clinically tested.

A number of different tests have been applied to the compounds, and will be explained and reported separately.

Uterotropic and Antiuterotropic Tests

The first group of tests to be reported were carried out to measure the compounds' ability to prevent the uterotropic effect caused by a potent estrogen, estradiol. The antiuterotropic effect, of course, is the fundamental indication of antiestrogenic activity. Many compounds having antiestrogenic activity also have estrogenic activity, and therefore the uterotropic effect of such compounds is always measured also. Estrogenic activity is a disadvantage in an antiestrogen, as is explained in the specification of the above-named patent application.

The test methods were explained in the specification. In general, each test was carried out by administering the test compound daily for three days, along with untreated control animals and animals to which estradiol was administered subcutaneously. On the fourth day, the animals were sacrificed, and the uteri were removed, freed of extraneous tissue and blotted dry with paper towels. The uteri were weighed to determine the uterotropic effect. In the antiuterotropic tests, the compound to be tested was administered concomitantly with subcutaneous

estradiol and the animals were sacrificed and the uteri weighed as above.

In the tables below, tests which have a close relationship to each other are grouped, and means of tests are calculated where it is appropriate to do so. All of the data is shown in the form of pairs of tables which may be compared with each other, one table for compound II and one table for compound I.

The column headed "Assay" gives the number of the test in which each line of data was generated, so that the reader can see which data came from the same test. The column headed N gives the number of animals in the test group, and the weights of the uteri in each group are shown as the mean, followed by the standard error. A mean of means is calculated where a number of tests used the same conditions. Doses of compounds are presented as micrograms per day, and organ weights are in milligrams.

In all cases, the results for control animals are immediately followed by the treatment results which are to be compared with those controls.

Table I

UTEROTROPIC AND ANTIUTEROTROPIC ACTIVITY OF TEST COMPOUNDS WHEN ADMINISTERED ORALLY TO IMMATURE RATS

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	774		6	24.4	1.38	24.0
	776		6	26.2	2.31	
	780		6	24.4	.59	
	783		6	23.5	.54	
	784		6	21.3	1.49	
Estradiol 0.1 µg (s.c.)	775		6	82.7	8.62	68.8
	777		6	65.6	4.72	
	780		6	75.3	7.06	
	783		6	60.8	2.86	
	784		6	59.5	5.02	

Table I (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound II	774	1000 µg	6	38.8	0.78	36.2
	776	"	6	40.8	1.26	
	780	"	6	37.6	0.88	
	784	"	6	27.5	1.05	
	774	100 µg	6	42.4	2.60	40.7
	776	"	6	48.6	2.17	
	780	"	6	39.4	3.20	
	784	"	6	32.3	2.68	
	774	10 µg	6	51.4	1.65	46.2
	776	"	6	48.9	2.33	
	780	"	6	48.9	1.51	
	784	"	6	35.7	1.98	
	780	1 µg	6	53.8	4.89	45.2
	784	"	6	36.5	1.42	
Estradiol 0.1 µg (s.c.) + Compound II	775	1000 µg	6	42.5	1.63	39.6
	777	"	6	41.7	1.77	
	780	"	6	36.9	1.49	
	783	"	6	37.1	2.25	
	775	100 µg	6	49.9	1.31	44.5
	777	"	6	44.8	2.31	
	780	"	5	37.6	3.52	
	783	"	6	45.8	1.57	
	775	10 µg	6	54.5	1.54	55.2
	777	"	6	54.6	1.94	
	780	"	6	50.2	1.38	
	783	"	6	61.5	1.53	
	780	1 µg	6	70.3	3.01	69.8
	783	"	6	69.3	3.11	

Compound I

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	783		6	23.5	0.5	23.9
	784		6	21.3	1.5	
	814		6	23.8	1.4	
	818		6	27.1	0.6	

Table I (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.)	783		6	60.8	2.9	61.0
	815		6	56.8	5.3	
	818		6	65.5	4.5	
Compound I	784	1000 µg	6	28.1	0.4	31.3
	814	"	6	34.4	1.5	
	784	100 µg	6	33.5	3.1	33.4
	814	"	6	33.2		
	784	10 µg	6	33.3	2.1	33.9
	814	"	6	34.4	1.8	
	784	1 µg	6	43.8	0.9	39.2
	814	"	6	34.5	1.8	
Estradiol 0.1 µg (s.c.) + Compound I	783	5000 µg	6	41.4	4.1	41.4
	783	1000 µg	6	45.6	2.5	34.3
	785	"	6	27.3	1.6	
	815	"	6	30.6	2.3	
	818	"	6	33.6	1.3	
	783	100 µg	6	36.8	1.9	32.9
	785	"	6	29.8	2.2	
	815	"	6	31.3	1.1	
	818	"	6	33.5	1.9	
	783	10 µg	6	40.4	2.8	41.4
	785	"	6	39.5	1.3	
	815	"	6	46.7	4.7	
	818	"	6	38.9	2.3	
	783	1 µg	6	63.9	2.6	53.7
	785	"	6	41.9	2.8	
	815	"	6	55.0	2.6	
	818	"	6	53.9	3.1	

Table II

ANTIUTEROTROPIC ACTIVITY OF TEST COMPOUNDS WHEN ADMINISTERED SUBCUTANEOUSLY WITH VARIOUS DOSES OF ESTRADIOL IN IMMATURE RATS

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	762		6	24.1	1.43	23.7
	765		6	23.2	2.24	
Estradiol 10 µg (s.c.)	762		6	103.5	2.38	103.3
	765		6	103.1	2.83	
Estradiol 10 µg (s.c.) + Compound II	765	1000 µg	6	41.3	1.25	41.3
	765	100 µg	6	55.8	2.69	55.8
	762	10 µg	6	72.3	3.86	72.3
Control	759		6	28.9	2.62	28.1
	761		6	25.6	1.66	
	764		6	29.9	1.90	
Estradiol 1 µg (s.c.)	759		6	88.7	6.70	90.0
	761		6	99.1	5.62	
	764		6	82.1	4.67	
Estradiol 1 µg (s.c.) + Compound II	764	1000 µg	6	39.2	1.31	39.2
	759	100 µg	6	49.3	2.69	48.6
	761	"	6	47.9	1.61	
	759	10 µg	6	59.9	3.04	
	761	"	6	61.2	2.70	60.6
	759	1 µg	6	76.0	6.13	76.0
Control	760		6	26.5	0.70	27.4
	764		6	29.9	1.90	
	782		6	21.1	1.65	
	786		6	26.7	1.73	
	866		6	24.6	1.30	
	868		6	43.1	1.54	
	880		6	27.9	1.61	
	889		6	31.3	3.38	
	892		6	27.4	1.74	
	897		6	23.8	2.26	
	918		6	27.9	2.93	
	925		6	22.4	2.91	
	953		6	23.5	1.90	

Table II (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.)	760		6	74.4	6.41	72.7
	764		6	88.3	7.39	
	782		6	71.5	3.96	
	786		6	78.9	4.93	
	866		5	47.1	4.70	
	868		6	74.7	5.34	
	880		6	72.1	5.07	
	889		6	61.8	9.29	
	892		6	89.2	9.61	
	897		6	66.6	6.97	
	918		6	71.8	9.38	
	925		6	70.9	5.07	
	917		6	71.8	9.38	
	953		6	78.6	3.15	
Estradiol 0.1 µg (s.c.) + Compound II	764	1000 µg	6	37.9	1.61	39.7
	782	"	6	33.6	1.46	
	786	"	6	36.8	1.99	
	866	"	6	33.9	0.45	
	868	"	5	40.3	0.83	
	880	"	6	49.0	2.90	
	889	"	6	50.8	4.00	
	892	"	6	40.2	2.11	
	897	"	6	40.9	2.22	
	918	"	3	36.7	3.60	
	925	"	5	41.1	0.75	
	954	"	6	38.6	1.90	
	760	100 µg	6	44.4	2.03	45.2
	868	"	6	41.1	2.43	
	880	"	6	54.5	5.27	
	889	"	6	44.8	2.27	
	892	"	6	39.5	2.53	
	897	"	6	46.6	2.11	
	760	10 µg	6	50.8	1.65	51.7
	868	"	6	47.0	1.19	
	880	"	6	56.0	2.44	
	889	"	6	51.6	3.25	
	892	"	6	50.8	4.25	
	897	"	6	54.1	4.09	
	868	1 µg	6	60.4	5.13	62.8
	880	"	6	64.4	4.21	
	889	"	6	60.3	3.81	
	892	"	6	66.2	7.10	
	897	"	6	62.5	2.25	

Table II (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	808		6	26.8	1.79	25.8
	812		6	28.0	2.86	
	820		6	22.5	1.57	
Estradiol 0.08 µg (s.c.)	808		6	53.7	8.98	47.9
	812		6	32.3	2.10	
	820		6	57.6	4.84	
Estradiol 0.08 µg (s.c.) + Compound II	808	1000 µg	6	35.5	0.83	37.5
	812	"	6	42.1	0.66	
	820	"	6	34.9	2.21	
	808	100 µg	6	39.4	1.46	40.5
	812	"	6	42.8	2.19	
	820	"	6	39.3	2.08	
	808	10 µg	6	39.3	1.39	42.9
	812	"	6	44.5	2.06	
	820	"	6	44.9	0.80	
	808	1 µg	6	50.4	3.89	49.5
	812	"	6	48.3	1.96	
	820	"	6	49.9	0.37	
Control	806		6	30.0	1.27	24.6
	809		6	23.0	1.47	
	821		6	20.7	2.00	
Estradiol 0.05 µg (s.c.)	806		6	50.9	1.72	51.5
	809		6	50.4	5.97	
	821		6	53.3	6.40	
Estradiol 0.05 µg (s.c.) + Compound II	806	1000 µg	6	38.3	1.53	37.8
	809	"	6	38.3	1.80	
	821	"	6	37.3	1.95	
	806	100 µg	6	45.9	1.53	42.5
	809	"	6	41.1	1.94	
	821	"	6	40.6	2.20	
	806	10 µg	6	49.9	2.27	45.4
	809	"	6	40.7	2.45	
	821	"	6	45.7	3.29	
	806	1 µg	6	53.5	3.82	54.7
	809	"	6	52.8	1.76	
	821	"	6	57.9	2.83	

Table II (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	806		6	30.0	1.27	27.8
	823		6	25.6	3.30	
Estradiol 0.03 µg (s.c.)	806		6	44.9	4.13	42.8
	823		6	40.6	3.62	
Estradiol 0.03 µg (s.c.) + Compound II	806	1000 µg	6	36.5	0.99	37.8
	823	"	6	39.1	1.26	
	806	100 µg	6	38.9	1.29	40.9
	823	"	6	42.9	3.23	
	806	10 µg	6	48.4	1.44	46.9
	823	"	6	45.4	1.46	
	806	1 µg	6	56.4	1.12	52.8
	823	"	6	49.1	2.80	

Compound I

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	810		6	24.8	1.2	24.8
Estradiol 10 µg (s.c.)	810		6	113.2	5.3	113.2
Estradiol 10 µg (s.c.) + Compound I	810	1000 µg	6	33.1	1.2	33.1
	810	100 µg	6	48.9	3.5	48.9
	810	10 µg	6	60.2	3.7	60.2
	810	1 µg	6	92.7	6.5	92.7
Estradiol 1 µg (s.c.)	810		6	84.4	5.8	84.4
Estradiol 1 µg (s.c.) + Compound I	810	1000 µg	6	30.2	0.9	30.2
	810	100 µg	6	33.7	0.8	33.7
	810	10 µg	6	42.2	2.3	42.2
	810	1 µg	6	71.9	3.4	71.9

Table II (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	782		6	21.1	1.7	24.7
	786		6	26.7	1.7	
	789		6	27.5	1.8	
	795		6	24.6	1.3	
	953		6	23.5	1.9	
Estradiol 0.1 µg (s.c.)	782		6	71.5	4.0	70.7
	786		6	79.0	4.9	
	789		6	71.4	5.4	
	795		6	76.4	4.5	
	866		5	47.1	4.7	
	953		6	78.6	3.2	
Estradiol 0.1 µg (s.c.) + Compound I	782	1000 µg	6	28.0	1.3	30.0
	786	"	6	28.3	1.8	
	789	"	6	31.6	1.5	
	795	"	6	27.6	1.4	
	866	"	6	32.4	0.6	
	918	"	3	30.2	1.4	
	954	"	6	31.7	1.4	
	782	100 µg	6	30.7	1.2	32.6
	786	"	6	35.2	0.9	
	789	"	6	33.0	2.6	
	795	"	6	31.3	0.9	
	782	10 µg	6	33.7	1.4	36.7
	786	"	6	33.5	1.3	
	789	"	6	41.2	1.8	
	795	"	6	38.4	2.2	
	782	1 µg	6	53.6	3.4	48.7
	786	"	6	47.6	4.2	
	789	"	6	45.9	1.7	
	795	"	6	47.7	3.2	
Control	808		6	26.8	1.8	25.8
	812		6	28.0	2.9	
	820		6	22.5	1.6	
Estradiol 0.08 µg (s.c.)	808		6	53.7	9.0	47.9
	812		6	32.3	2.1	
	820		6	57.6	4.8	

Table II (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.08 µg (s.c.) + Compound I	808	1000 µg	6	30.9	1.6	33.5
	812	"	6	36.8	0.7	
	820	"	6	32.8	0.9	
	808	100 µg	6	31.5	1.1	33.9
	812	"	6	35.1	0.8	
	820	"	6	35.0	1.1	
	808	10 µg	6	33.4	1.8	35.5
	812	"	6	33.4	1.8	
	820	"	6	39.6	2.2	
	808	1 µg	6	44.1	2.3	44.0
	812	"	6	43.3	2.3	
	820	"	6	44.7	1.6	
Control	806		6	30.0	1.3	24.6
	809		6	23.0	1.5	
	821		6	20.7	2.0	
Estradiol 0.05 µg (s.c.)	806		6	50.9	1.7	51.5
	809		6	50.4	6.0	
	821		6	53.3	6.4	
Estradiol 0.05 µg (s.c.) + Compound I	806	1000 µg	6	36.2	2.2	33.9
	809	"	6	32.0	1.4	
	821	"	6	33.6	0.8	
	806	100 µg	6	36.5	1.9	35.3
	809	"	6	33.8	2.1	
	821	"	6	35.7	1.4	
	806	10 µg	6	41.0	1.8	37.8
	809	"	6	36.5	2.6	
	821	"	6	35.8	1.9	
	806	1 µg	6	49.9	2.7	49.5
	809	"	6	49.3	2.7	
	821	"	6	49.9	1.5	

Table II (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	806		6	30.0	1.3	27.8
	823		6	25.6	3.3	
Estradiol 0.03 µg (s.c.)	806		6	44.9	4.1	42.8
	823		6	40.6	3.6	
Estradiol 0.03 µg (s.c.) + Compound I	806	1000 µg	6	31.0	1.7	32.3
	823	"	6	33.5	2.4	
	806	100 µg	6	33.7	2.0	33.4
	823	"	6	33.0	0.9	
	806	10 µg	6	35.8	1.9	34.3
	823	"	6	32.8	1.4	
	806	1 µg	6	42.5	2.8	43.5
	823	"	6	44.5	3.5	

Table III

UTEROTROPIC ACTIVITY OF SUBCUTANEOUSLY ADMINISTERED TEST COMPOUNDS IN IMMATURE RATS

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	760		6	26.5	0.70	25.6
	761		6	25.6	1.66	
	764		6	29.9	1.90	
	765		6	23.2	2.24	
	779		6	22.7	0.79	
	854		6	28.7	2.13	
	856		6	29.0	1.76	
	865		6	24.6	1.30	
	867		6	28.9	2.55	
	872		6	21.7	0.80	
	891		6	27.4	1.74	
	917		6	27.9	2.93	
	924		6	22.4	2.91	
	932		6	21.3	1.23	
	953		6	23.5	1.90	

Table III (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound II	764	1000 µg	6	43.2	2.04	39.1
	765	"	6	43.2	1.55	
	779	"	6	35.8	1.37	
	854	"	6	42.9	2.77	
	856	"	6	33.6	2.32	
	865	"	6	38.5	1.14	
	867	"	6	33.4	0.60	
	872	"	6	45.7	2.42	
	891	"	6	41.5	3.26	
	917	"	3	43.6	2.87	
	924	"	6	35.8	1.76	
	932	"	6	34.0	1.57	
	953	"	6	36.9	0.48	
	758	100 µg	6	37.9	1.79	42.9
	760	"	6	47.4	2.82	
	761	"	6	42.4	1.29	
	779	"	6	42.0	2.20	
	872	"	6	44.2	2.47	
	891	"	6	43.4	2.28	
	760	10 µg	6	48.8	1.42	46.5
	761	"	6	44.4	1.64	
	779	"	6	46.6	2.49	
	872	"	6	49.1	2.43	
	891	"	6	43.6	2.68	
	779	1 µg	6	55.7	1.71	47.5
	872	"	6	49.5	2.31	
	891	"	6	37.3	2.47	

Compound I

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	779		6	22.7	0.8	25.2
	792		6	23.0	1.4	
	865		6	24.6	1.3	
	867		6	28.9	2.6	
	901		6	31.7	2.7	
	908		6	21.5	1.4	
	917		4	24.3	3.0	
Compound I	779	1000 µg	6	36.8	1.1	33.6
	792	"	6	33.9	0.9	
	865	"	6	33.9	1.3	
	867	"	6	32.8	1.4	
	901	"	6	32.0	2.9	
	917	"	3	34.1	3.5	
	954	"	6	31.6	1.5	

Table III (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound I	779	100 µg	6	41.0	1.4	37.1
	792	"	6	35.9	1.3	
	901	"	6	34.3	1.1	
	779	10 µg	6	45.9	1.8	40.9
	792	"	6	38.4	1.9	
	901	"	6	38.5	0.8	
	779	1 µg	6	49.6	3.2	45.0
	792	"	6	47.7	2.2	
	901	"	6	37.7	1.9	

Table IV

UTEROTROPIC AND ANTIUTEROTROPIC ACTIVITY OF TEST COMPOUNDS WHERE ADMINISTERED SUBCUTANEOUSLY TO ADULT OVARIECTOMIZED MICE

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	772		10	28.0	1.08	27.5
	773		10	27.3	1.08	
	877		8	27.3	1.14	
Estradiol 0.1 µg (s.c.)	773		10	94.5	1.08	109.1
	877		6	123.6	8.35	
Compound II	772	1000 µg	10	59.5	1.73	59.5
	772	100 µg	10	53.2	4.13	53.2
	772	10 µg	10	62.9	5.47	62.9
	772	1 µg	10	42.4	2.09	42.4
	772	0.1 µg	10	24.5	1.34	24.5
	772	0.01 µg	10	24.8	1.13	24.8

Table IV (cont'd)

Compound II (cont'd)

	Assay	Dose of Compound II Per day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.)	773	1000 µg	10	48.0	3.05	49.3
	877	"	6	50.5	2.10	
+ Compound II	773	100 µg	10	50.0	2.35	50.0
	773	10 µg	10	61.8	5.68	61.8
	773	1 µg	9	92.3	7.35	92.3
	773	0.1 µg	10	90.9	5.53	90.9

Compound I

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	801		10	12.3	0.4	12.3
Compound I	801	1000 µg	10	23.1	1.5	23.1
	801	100 µg	10	24.8	0.8	24.8
	801	10 µg	10	26.2	1.3	26.2
	801	1 µg	10	25.6	1.1	25.6
	801	0.1 µg	10	18.2	1.0	18.2
Estradiol 0.1 µg (s.c.)	801		10	65.9	4.1	65.9
Estradiol 0.1 µg (s.c.) + Compound I	801	1000 µg	6	21.3	1.1	21.3
	801	100 µg	6	22.1	1.3	22.1
	801	10 µg	6	31.1	1.1	31.1
	801	1 µg	6	48.7	3.1	48.7
	801	0.1 µg	6	54.9	2.8	54.9
Control	915		6	26.2	2.9	26.2
Compound I	915	1000 µg	6	38.9	2.0	38.9
	915	100 µg	6	42.5	2.0	42.5
	915	10 µg	6	48.3	3.2	48.3
	915	1 µg	6	36.5	4.0	36.5
	915	0.1 µg	5	31.1	2.2	31.1
Estradiol 0.3 µg (s.c.)	915		6	122.4	15.9	122.4
Estradiol 0.3 µg (s.c.) + Compound I	915	1000 µg	6	40.2	1.4	40.2
	915	100 µg	6	40.4	2.0	40.4
	915	10 µg	6	66.2	6.6	66.2
	915	1 µg	6	97.7	6.7	97.7
	915	0.1 µg	5	103.7	7.8	103.7

Table V

UTEROTROPIC AND ANTIUTEROTROPIC ACTIVITY OF SUBCUTANEOUSLY ADMINISTERED TEST COMPOUNDS TO IMMATURE MICE

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	685		10	7.6	0.40	12.8
	740		10	15.9	0.82	
	744		10	18.1	0.77	
	770		10	10.9	0.52	
	771		10	11.4	0.78	
Compound II	770	1000 µg	10	23.5	1.43	23.5
	685	100 µg	10	21.7	1.74	22.4
	767	"	10	21.2	0.80	
	770	"	10	24.2	0.98	
	767	10 µg	10	22.5	0.97	24.6
	770	"	10	26.7	1.55	
	767	1 µg	10	23.9	1.09	25.8
	770	"	10	27.7	2.05	
	770	0.1 µg	10	14.5	1.27	14.5
	770	0.01 µg	10	9.9	0.50	9.9
Estradiol 0.1 µg (s.c.)	740		10	59.3	3.02	54.8
	744		10	52.6	1.51	
	771		10	52.6	1.92	
Estradiol 0.1 µg (s.c.) + Compound II	771	1000 µg	10	21.1	0.61	21.1
	740	100 µg	10	25.8	1.06	23.5
	744	"	10	24.1	1.69	
	771	"	10	20.7	0.68	
	740	10 µg	10	27.4	1.02	27.4
	744	"	10	30.9	1.86	
	771	"	10	23.9	1.20	
	740	1 µg	10	35.7	2.20	43.4
	744	"	10	50.1	2.95	
	771	"	10	44.5	1.97	
	771	0.1 µg	10	51.1	1.67	51.1
	771	0.01 µg	10	52.1	1.67	52.1

Table V (cont'd)

Compound I

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	790		10	10.6	0.7	10.6
Compound I	790	1000 µg	10	14.6	0.7	14.6
	790	100 µg	10	19.3	0.9	19.3
	790	10 µg	10	23.9	1.1	23.9
	790	1 µg	10	22.9	1.1	22.9
	790	0.1 µg	10	19.3	1.5	19.3
	790	0.01 µg	10	19.0	1.0	19.0
Estradiol 0.1 µg (s.c.)	790		10	50.3	2.2	10.6
Estradiol 0.1 µg (s.c.) + Compound I	791	1000 µg	10	13.9	0.8	13.9
	791	100 µg	10	14.6	0.6	14.6
	791	10 µg	10	18.9	0.7	18.9
	791	1 µg	10	26.5	1.8	26.5
	791	0.1 µg	10	40.6	2.0	40.6
	791	0.01 µg	10	41.3	1.9	41.3

Table VI

THE ANTIUTEROTROPIC ACTIVITY OF SUBCUTANEOUSLY ADMINISTERED TEST COMPOUNDS AS HCl SALTS IN IMMATURE RATS

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	897		6	23.8	2.26	23.8
Estradiol 0.1 µg (s.c.)	897		6	66.6	6.97	66.6
Estradiol 0.1 µg (s.c.) + Compound II	897	1000 µg	6	38.8	1.30	38.8
	897	100 µg	6	42.1	1.25	42.1
	897	10 µg	6	50.7	2.71	50.7
	897	1 µg	6	66.0	2.97	66.0

Compound I

Control	873		6	21.7	0.8	26.8
	880		6	27.9	1.6	
	889		6	31.3	3.4	
	899		6	31.9	4.7	
	933		6	21.3	1.2	

Table VI (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.)	873		6	74.3	5.8	71.1
	880		6	72.1	5.1	
	889		6	61.8	9.3	
	899		6	79.6	7.0	
Estradiol 0.1 µg (s.c.) + Compound I	873	1000 µg	6	34.0	1.2	37.2
	880	"	6	46.5	3.5	
	889	"	6	39.1	1.3	
	899	"	6	33.6	1.6	
	873	100 µg	6	35.5	0.9	40.6
	880	"	6	43.8	1.1	
	889	"	6	44.5	1.6	
	899	"	6	35.5	1.8	
	873	10 µg	6	44.1	3.1	48.6
	880	"	6	53.5	2.6	
	889	"	6	46.9	4.4	
	899	"	6	49.8	3.0	
	873	1 µg	6	66.7	2.2	63.7
	880	"	6	70.9	3.3	
	889	"	6	59.1	7.7	
	899	"	6	58.2	5.4	

A great many comparisons are presented above, using compounds I and II at administration rates from 0.01 microgram per day to 1000 micrograms per day, and challenging the effect of the compounds with a number of different doses of estradiol. It is my conclusion, based on my study of all of the tests, that compound I, when administered in an optimal dose, is ultimately capable of controlling almost 90 percent of the uterotropic effect of estradiol. Compound II, when also administered in an optimal manner, is ultimately capable of inhibiting only about 75 percent of the effect of estradiol.

Compound I is effective at much lower administration rates than is compound II. In general, I conclude that a given antiestrogenic effect obtained by the administration of 1000 micrograms per day of compound II can be matched by the administration of only 10 micrograms per day of compound I.

I conclude that compound I is less uterotropic than is compound II. Neither compound has very notable uterotropic activity, and both compounds have the strange characteristic of causing less uterotropic response when administered at high doses than they cause at low doses. The maximum uterotropic response produced by compound II is clearly greater than the maximum response caused by compound I, under any test conditions.

Duration of Action Tests

The tests reported here were carried out to determine the length of the anti-estrogenic action of compounds I and II, when a single subcutaneous dose of each compound is administered.

Each test was begun by administering one injection of the designated dose of a compound to ovariectomized rats. Various periods of time were allowed to pass, and then the treated rats were each injected with 0.3 microgram of estradiol benzoate (EB) per day for 3 days, and the rats were sacrificed and the uteri weighed on the day after the third injection. The times between administration of the test compound and the onset of estradiol benzoate administration ranged up to 90 days, as shown in the table below.

The results are tabulated in Table VII; the headings are as explained above.

Table VII

THE DURATION OF ANTIESTROGENIC ACTION OF TEST COMPOUNDS
FOLLOWING A SINGLE INJECTION IN OVARIECTOMIZED RATS

Compound II

	Assay	Dose of Compound II Per Day	N	Mean Uterine Weight (mg)	Percent of EB Response	Mean of Means Uterine Weight	Mean of Means Percent of EB
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 0-2	946	1000 µg	6	49.7	40.9	49.7	40.9
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 3-5	946	1000 µg	6	46.1	35.6	46.1	35.6
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 5-7	946	1000 µg	6	52.7	45.0	52.7	45.0
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 10-12	946	1000 µg	6	64.4	61.4	64.4	61.4
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 20-22	946	1000 µg	6	106.3	120.0	106.3	120.0
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 30-32	946	1000 µg	6	100.5	112.1	100.5	112.1
Compound II x 1 Day 0 EB 0.3 µg x 3 Day 60-62	946	1000 µg	6	111.4	127.3	111.4	127.3

Table VII (cont'd)

	Compound I						
	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Percent of EB Response	Mean of Means Uterine Weight	Mean of Means Percent of EB
Control	941		6	29.2		23.9	
	946		6	20.6			
	959		6	21.9			
Estradiol Benzoate 0.3 µg per Day x 3	941		6	110.7		105.8	
	946		6	91.9			
	959		6	114.9			
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 0-2	941	1000 µg	6	61.7	39.9	56.4	35.6
	959	"	6	51.0	31.3		
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 3-5	941	1000 µg	6	64.7	43.6	59.3	39.0
	959	"	6	53.9	34.4		
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 5-7	941	1000 µg	6	73.2	54.0	65.5	46.3
	959	"	6	57.8	38.6		
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 10-12	941	1000 µg	6	60.9	38.9	65.1	45.0
	959	"	6	69.3	51.0		

Table VII (cont'd)

Compound I (cont'd)

	Assay	Dose of Compound I Per Day	N	Mean Uterine Weight (mg)	Percent of EB Response	Mean of Means Uterine Weight	Mean of Means Percent of EB
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 20-22	941	1000 µg	6	70.3	50.4	70.0	50.9
	959	"	6	69.7	51.4		
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 30-32	941	1000 µg	6	76.2	57.7	76.2	57.7
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 45-47	950	1000 µg	6	84.6	68.0	84.6	68.0
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 60-62	950	1000 µg	6	85.3	68.8	85.3	68.8
Compound I x 1 Day 0 EB 0.3 µg x 3 Day 90-92	950	1000 µg	6	110.2	99.4	110.2	99.4

These experiments clearly show a rather remarkable difference between compounds I and II. A single injection of either compound reduced the uterotropic response of estradiol benzoate, when the estrogen was administered on the same day as the test compound. As the interval between administration of the test compound and the estrogen increased, the antiestrogenic effect of the test compound gradually disappeared. The uterine response to estradiol benzoate was recovered fully 20 days after administration of compound II, while full recovery after administration of compound I required 90 days.

It is very clear that compound I continues to be active in the animals for a much longer time than does compound II.

Relative Binding Affinity Tests

Relative binding affinity tests of compounds I and II were carried out according to the test explained in the specification of the above-named patent application. The procedure is lengthy and will not be repeated; it is an in vitro test carried out on uterine cytosol which determines the affinity of an antiestrogenic compound for estrogen receptor, relative to the affinity of estradiol. The corresponding value for estradiol, to which the experimental results are compared, is by definition 1.0.

Table VIII below reports tests in which tissue was obtained from female immature laboratory rats.

Table VIII

RELATIVE BINDING AFFINITY OF TEST COMPOUNDS FOR RAT UTERINE CYTOSOL ESTROGEN RECEPTORS UNDER VARIOUS CONDITIONS

Compound II

Assay	4°,1 hr	4°,2 hr	4°,24 hr	15°,1 hr	30°,30 min
985	.69				
1120				.71	
1123	.47				
1124				.86	
1131					1.25
1135	.73				
1136				.61	
1313	.19				
1335	.25				2.70
1338	1.00			1.20	1.20
1340	.76			.66	
1344	.48			.38	.38
1346	.66			.60	.10
1349	.47			.59	2.72
1350	.95			.61	.95
1352				.96	
1354	.36			.66	1.00
1355	.60		.46		
1358	.19		.47		
1359				.45	.46
1362					.46
1363	.40		.46		
1366					.60
1377	.43			.23	.74
1377	.50			.72	.92
1379	.31			.31	.34
1383	.36		.37		
1386				1.84	1.00
1387	.47				
1403	.58			.68	1.70
1405					.92
1419				1.86	
1421	.83		.87		
1431				.81	
1447	.40		.85	.75	1.50
1457	1.04			1.00	2.25
1460	.24		.21	.25	.72
1460	.21		.43	.33	.79
1465	.88		1.00	1.50	2.20
1550		.74		.90	1.64
Mean	0.54	.74	0.57	0.78	1.15
Standard Error	±0.05	0	±0.09	±0.09	±0.15

Table VIII (cont'd)

Compound I

Assay	4°,1 hr	4°,2 hr	4°,24 hr	15°,1 hr	30°,30 min
1447	.02		.45	.97	1.48
1456	.62		1.03	.61	1.24
1463	1.70		1.90	1.90	2.30
1464	3.70		1.20	1.70	2.50
1465	1.10		1.50	1.90	3.80
1485	2.67		2.00	1.90	7.25
1512	0.83		1.30	.91	1.90
1514	1.30			4.20	1.20
1514	0.94			.91	3.20
1550		1.10		1.00	2.35
1557		2.60		3.79	4.57
Mean	1.43	1.85	1.34	1.80	2.90
Standard Error	± .38		± .20	± .36	± .54

Table IX below reports relative binding affinity tests in which the tissue was obtained from laboratory mice.

Table IX

THE RELATIVE BINDING AFFINITY OF TEST COMPOUNDS FOR MOUSE UTERINE CYTOSOL ESTROGEN RECEPTORS UNDER VARIOUS CONDITIONS

Compound II

Assay	4°,1 hr	4°,24 hr	15°,1 hr	30°,30 min
		Immature Mice		
1469	.38	.25	.30	.34
		Adult Ovariectomized Mice		
1467	.72	.83	.89	1.43
1475	.82	2.94	3.01	5.26
Mean	.77	1.89	1.95	3.35

Compound I

Assay	4°,1 hr	4°,24 hr	15°,1 hr	30°,30 min
		Immature Mice		
1469	1.10	1.30	1.10	2.20
		Adult Ovariectomized Mice		
1467	0.38	0.37	2.40	1.92
1475	1.27	2.48	3.73	4.76
Mean	0.83	1.43	3.07	3.34

The data above obtained from rat tissue very clearly show that compound I has much greater affinity for estrogen receptors than does compound II. The data show that the relative binding affinity of each compound increases with increasing temperature. Both compounds have maximal relative binding affinity at 30°, at which condition compound II's affinity is 1.15. In contrast, at 30°, compound I shows an affinity of 2.9 times the affinity of estradiol. The lowest affinity achieved by compound I (at 4°) is 1.34, greater than the highest affinity achieved by compound II.

The in vitro affinity studies are believed to show the relative ability of the antiestrogenic compounds to occupy estrogen receptors in vivo to the exclusion of endogenous estrogen, thereby reducing estrogen-induced responses in the target organs. The relative binding affinities of compounds I and II indicate to me that compound I has at least twice the affinity for receptor of compound II, under the various assay conditions which have been used, and that it can therefore be expected to have much more ability to exclude endogenous estrogen from the target organs.

Only a few tests with mouse tissue have been done. No firm conclusions can be drawn from these few tests, but the results do not seem to contradict my conclusion from the rat tissue tests.

Dissociation Tests

Tests were carried out according to the procedure of Test 5 in the specification of the above-named patent application to determine the rate at which compounds I and II dissociated in vitro from rat uterine cytosol estrogen receptors. The dissociation test method is designed to estimate the rate at which

the compounds are dissociated from estrogen receptors and replaced by estradiol, after having been once bound to the receptors. The test method in use, as described in the specification, depends on actual measurement of receptor bound radiolabeled estradiol through an exchange process, because of the lack of labeled samples of compounds I and II, and to that extent they are indirect measurements.

The following table reports dissociation measurements for compounds I and II at two temperatures, relative to comparable measurements for estradiol itself.

Table X

THE DISSOCIATION OF TEST COMPOUNDS FROM RAT UTERINE CYTOSOL ESTROGEN RECEPTORS AT 30°C

(Relative Percent Bound)

Assay	1 min	5 min	15 min	30 min	1 hr	2 hr
			Estradiol			
1477	67.4	56.5	28.8	20.1	6.3	18.6
1478	63.1	45.0	45.7	46.9	24.4	42.6
Mean	65.3	50.8	37.3	33.5	15.4	30.6
			Compound II			
1437	100.0	100.0	100.0	77.8	100.0	89.5
1446	63.4	47.5	12.6	8.5	28.9	6.9
1450	58.8	70.8	51.5	64.4	75.3	43.0
1477	58.2	49.7	28.8	21.3	53.5	45.9
1478	82.3	59.3	53.2	54.8	36.8	54.2
1484	87.9	77.4	65.6	62.8	36.0	33.8
1486	87.1	92.7	73.0	58.1	42.4	40.9
Mean	76.8	71.1	55.0	49.7	53.3	44.9
Standard Error	± 6.26	± 7.71	±10.87	± 9.48	± 9.70	± 9.34

Table X (cont'd)

Assay	1 min	5 min	15 min	30 min	1 hr	2 hr
			Estradiol			
1478	63.1	45.0	45.7	46.9	24.4	42.6
1502	67.2	38.1	40.8	22.8	9.9	21.5
1504	50.9	44.6	34.6	33.9	24.1	4.7
1506	52.3	24.3	18.5	13.9	40.5	0
1509	87.1	63.6	36.6	32.7	14.7	14.2
1510	61.8	48.3	23.7	18.3	5.8	1.3
1511	63.9	54.1	17.9	24.9	20.9	23.8
Mean	63.8	45.4	31.1	27.6	20.0	15.4
Standard Error	± 4.52	± 4.67	± 4.19	± 4.21	± 4.33	± 5.77
			Compound I			
1478	61.0	70.2	49.7	26.3	2.7	18.6
1502	66.9	56.9	42.8	19.6	16.1	26.3
1504	52.6	67.2	67.6	57.1	54.8	53.1
1506	79.2	53.1	46.9	48.7	27.3	0
1509	75.9	70.8	54.7	47.8	38.1	40.3
1510	49.9	53.6	34.1	24.4	19.9	24.1
1511	51.9	57.7	42.9	38.1	32.9	20.3
Mean	62.55	61.4	48.4	37.4	27.4	26.1
Standard Error	± 4.50	± 2.94	± 4.02	± 5.42	± 6.34	± 6.37

THE DISSOCIATION OF TEST COMPOUNDS FROM RAT UTERINE CYTOSOL ESTROGEN RECEPTORS AT 4°C

Compound II

Assay	1 min	5 min	15 min	30 min	1 hr	2 hr
1453	68.1	55.6	26.4	63.9	39.7	44.8
1480	16.4	4.1	21.0	13.9	48.0	26.4
1488	71.4	73.2	62.6	63.8	70.1	64.6
1494	74.1	63.6	69.9	70.6	62.8	62.3
1495	68.5	77.7	58.8	68.6	59.4	55.6
1498	30.8	57.2	32.4	25.9	47.3	35.9
1500	79.2	66.8	70.9	64.6	74.9	55.8
Mean	58.4	56.9	48.9	53.0	57.5	49.3
Standard Error	± 9.22	± 9.30	± 8.12	± 8.71	± 4.89	± 5.37

Table X (cont'd)

Assay	1 min	5 min	15 min	30 min	1 hr	2 hr
			Estradiol			
1488	81.3	77.3	67.0	65.7	57.1	52.4
1495	72.6	75.8	62.7	56.9	61.4	62.9
1498	35.9	61.4	45.4	36.9	37.9	46.5
1501	26.3	70.6	85.1	69.7	74.5	67.6
1503	67.5	47.6	51.6	51.1	35.3	45.5
1505	51.1	79.8	84.6	76.1	92.8	76.9
1513	58.1	65.9	48.3	64.9	60.5	57.7
Mean	56.1	68.3	63.5	60.2	59.9	58.5
Standard Error	± 7.50	± 4.24	± 6.22	± 4.96	± 7.55	± 4.34
			Compound I			
1488	76.1	72.4	58.2	76.1	64.4	64.8
1498	19.6	70.2	47.0	48.4	49.1	41.3
1503	37.0	36.4	60.4	55.0	59.9	63.7
1505	67.9	67.9	93.2	79.9	76.8	61.5
1513	58.2	54.4	52.7	71.8	56.6	58.0
Mean	52.3	57.2	63.9	68.5	61.9	57.9
Standard Error	± 7.54	± 8.80	± 7.91	± 4.54	± 6.44	± 4.30

It is evident that the dissociation test method is extremely sensitive and subject to variation. The instances where the percent of compound bound to the receptor is higher at a later point than an earlier one indicate the problem. Therefore, small differences in results cannot be relied upon as meaningful. It can be concluded, however, that neither compound is significantly dissociated at 4° over a 24 hour period. At 30° it is apparent that both compounds I and II dissociate more slowly than estradiol. The data do not permit any conclusion about the dissociation rates of compound I and II relative to each other. The reason is that the estradiol standard curves in the two sets of experiments are quite different, where they exist at all. It should be noted, for example, that the mean percent bound of estradiol in the compound I tests at 2 hours is 15.4 percent, and

it is twice as great in the compound II tests. Hence, no definite conclusion is allowed by the data.

It is noted that test 1478 was the only one in which both compound I and compound II were tested. Its results indicate that compound II is less dissociated than compound I at most of the time intervals. That test, however, appears to be atypical, because in it compound I is more dissociated than estradiol, whereas it is less dissociated than estradiol in the tests as a whole.

Regression Tests

The objective of these tests was to determine if the compounds could regress uterine hypertrophy caused by estradiol, when the compounds were administered after hypertrophy was established.

The tests were begun by administering 0.1 microgram of estradiol to each immature ovariectomized rat each day for periods of from 3 to 13 days. Animals were sacrificed and the uteri removed and weighed on the day following the last injections.

Other groups of animals were administered estradiol for three days, and were then not treated for periods up to 10 days, and uterine weights were obtained at the end of the no-treatment periods.

The test compounds were administered to other groups of animals together with estradiol for periods of from 1 to 10 days, after estradiol alone had been administered for 3 days, and the uteri were harvested and weighed to assess the ability of the compounds to regress the uterotropic effect.

The results of the tests are shown in the following Table XI.

Table XI

INDUCED REGRESSION OF ESTRADIOL STIMULATED UTERINE HYPERTROPHY IN IMMATURE OVARIECTOMIZED RATS

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.) Day 1-3	811		6	97.4	8.30	92.6
	813		6	83.3	6.92	
	819		6	91.6	5.30	
	825		6	107.4	12.00	
	900		6	91.3	4.54	
	930		5	84.7	5.91	
Estradiol 0.1 µg (s.c.) Day 1-4	811		6	105.4	4.56	109.7
	813		6	119.4	2.22	
	825		6	102.3	8.66	
	903		6	111.8	14.40	
Estradiol 0.1 µg (s.c.) Day 1-6	811		6	105.2	9.70	109.9
	813		6	102.5	2.15	
	825		5	132.9	14.51	
	903		6	99.0	7.58	
Estradiol 0.1 µg (s.c.) Day 1-8	811		6	118.7	7.13	122.4
	813		6	124.7	8.38	
	825		6	125.8	4.49	
	903		6	112.8	8.43	
	930		5	129.8	10.39	
Estradiol 0.1 µg (s.c.) Day 1-13	819		6	138.6	8.47	138.6
Estradiol 0.1 µg (s.c.) Day 1-3--No Treatment Day 4	811		6	63.3	4.63	60.5
	813		6	54.8	2.20	
	843		6	59.4	2.67	
	903		6	64.4	2.23	
Estradiol 0.1 µg (s.c.) Day 1-3-- No Treatment Day 4-6	811		6	63.1	3.27	55.7
	813		6	46.4	3.02	
	843		6	53.9	5.44	
	903		6	59.3	3.67	
Estradiol 0.1 µg (s.c.) Day 1-3--No Treatment Day 4-8	811		6	54.0	2.34	45.1
	813		6	41.1	2.65	
	843		6	42.0	1.94	
	903		6	43.1	1.85	
Estradiol 0.1 µg (s.c.) Day 1-3--No Treatment Day 4-13	819		6	33.2	1.71	37.4
	849		6	41.5	3.36	

Table XI (cont'd)

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. II Day 4	811	1000 µg	6	72.2	3.95	73.4
	825	"	6	71.6	3.86	
	900	"	6	76.3	3.68	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. II Day 4-6	811	1000 µg	6	69.9	2.46	72.3
	825	"	6	69.9	2.95	
	900	"	6	77.0	2.83	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. II Day 4-8	811	1000 µg	6	63.1	3.77	68.2
	825	"	6	69.9	4.03	
	900	"	6	71.7	5.30	
	930	"	5	68.1	3.34	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. II Day 4-13	819	1000 µg	6	77.1	4.97	75.5
	822	"	6	73.9	5.71	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. I Day 4	825	1000 µg	6	61.1	2.35	61.3
	843	"	6	62.1	1.44	
	849	"	6	65.3	3.48	
	898	"	6	61.5	4.06	
	807	"	6	56.4	3.83	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. I Day 4-6	825	1000 µg	6	63.8	2.76	61.3
	843	"	6	57.6	3.39	
	849	"	6	66.5	3.20	
	898	"	6	61.4	5.91	
	807	"	6	57.0	4.60	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. I Day 4-8	825	1000 µg	6	46.9	2.19	53.5
	843	"	6	55.3	3.67	
	849	"	6	52.3	2.04	
	898	"	6	58.6	3.33	
	807	"	6	50.7	3.58	
	930	"	6	57.4	4.70	
Estradiol 0.1 µg (s.c.) Day 1-3--Estradiol + Comp. I Day 4-13	849	1000 µg	6	58.6	2.53	58.6

The tests show clearly that both of compounds I and II can regress the established uterotropic effect of estradiol, and that compound I is the more effective. At each of the four periods of administration of test compounds -- 1, 3, 5 and 10 days -- the animals treated with compound I showed less uterotropic effect than did the animals treated with compound II, demonstrating greater ability to regress estrogen-induced uterine hypertrophy.

Looking at the 5-day treatments (from 4 to 6 tests at each condition) the animals receiving no treatment but the initial 3 days of estrogen had mean uterine weight of 45.1 mg., the maximum possible regression. Animals receiving 5 additional days of estradiol had mean uterine weight of 120.5 mg. Those receiving estradiol plus compound II had mean weight of 68.2 mg., and those receiving estradiol plus compound I had mean weight of 53.5 mg. Thus, compound I regressed the uteri to within 8.4 mg. of the no-treatment level, but compound II regressed the uteri only to within 23.1 mg. of the no-treatment level.

Blockade Tests

These tests were carried out to determine the ability of the compounds to prevent the uterotropic effect of estradiol or tamoxifen (an anti-estrogenic drug which has a pronounced uterotropic effect), when the compounds were administered before the uterotropic agent. Immature ovariectomized rats were used, and were first treated by the injection of 1000 micrograms per day of the test compound for 3 to 8 days. Animals were sacrificed and the uteri weighed to establish the base line conditions. Then other animals were treated with the test compound daily for three days, followed by from 1 to 10 days of treatment with test compound plus estradiol, estradiol alone, or test compound plus tamoxifen. The results are in the following Table XII.

Table XII

BLOCKADE OF THE UTEROTROPIC ACTION OF ESTRADIOL OR TAMOXIFEN IN IMMATURE OVARIECTOMIZED RATS

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound II Day 1-3	816	1000 µg	6	43.8	1.70	43.4
	830	"	6	42.2	3.90	
	890	"	6	43.4	2.62	
	893	"	6	40.9	2.24	
	896	"	6	42.8	2.90	
	934	"	5	47.3	1.60	
Compound II Day 1-4	816	1000 µg	6	43.4	1.68	44.6
	831	"	6	45.7	2.63	
Compound II Day 1-6	816	1000 µg	5	44.8	1.83	45.2
	831	"	6	45.6	3.60	
Compound II Day 1-8	816	1000 µg	6	45.0	2.57	46.9
	831	"	6	48.7	1.47	
Compound II Day 1-3--Comp. II + E^2 0.1 µg (s.c.) Day 4	816	1000 µg	6	42.2	2.62	41.1
	831	"	6	41.7	1.62	
	896	"	6	39.4	1.74	
Compound II Day 1-3--Comp. II + E^2 0.1 µg (s.c.) Day 4-6	816	1000 µg	6	47.9	2.60	50.7
	831	"	6	54.6	2.63	
	896	"	6	49.6	1.79	
Compound II Day 1-3--Comp. II + E^2 0.1 µg (s.c.) Day 4-8	816	1000 µg	6	45.0	2.57	49.8
	831	"	6	51.7	0.99	
	896	"	6	50.7	1.84	
	934	"	6	51.6	2.22	
Compound II Day 1-3--E^2 0.1 µg (s.c.) Day 4	819	1000 µg	6	46.1	1.87	46.4
	830	"	6	46.6	3.24	
Compound II Day 1-3--E^2 0.1 µg (s.c.) Day 4-6	819	1000 µg	6	45.3	1.69	43.8
	830	"	6	42.2	2.12	
Compound II Day 1-3--E^2 0.1 µg (s.c.) Day 4-8	819	1000 µg	6	46.9	2.43	46.1
	830	"	6	45.3	1.30	

Table XII (cont'd)

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound II Day 1-3--E^2 0.1 µg (s.c.) Day 4-13	819	1000 µg	6	58.1	4.03	64.8
	830	"	6	71.5	6.29	
Compound II Day 1-3--Comp. II + Tamoxifen 1000 µg Day 4	836	1000 µg	6	57.0	4.80	56.7
	839	"	6	57.0	2.05	
	893	"	6	56.0	5.11	
Compound II Day 1-3--Comp. II + Tamoxifen 1000 µg Day 4-6	836	1000 µg	6	73.2	4.60	69.1
	839	"	6	69.4	4.47	
	893	"	6	64.8	5.91	
Compound II Day 1-3--Comp. II + Tamoxifen 1000 µg Day 4-8	836	1000 µg	6	71.4	4.02	72.7
	839	"	6	76.4	5.95	
	893	"	6	70.2	4.89	
Compound I Day 1-3	822	1000 µg	6	39.4	2.32	41.3
	845	"	6	44.2	2.41	
	849	"	6	35.0	2.68	
	903	"	6	45.5	2.98	
	934	"	6	42.6	1.68	
Compound I Day 1-4	845	1000 µg	6	38.2	2.14	39.6
	849	"	6	40.9	1.72	
Compound I Day 1-6	845	1000 µg	6	41.3	1.72	44.4
	849	"	6	47.5	3.15	
Compound I Day 1-8	854	1000 µg	6	41.5	1.77	42.6
	849	"	6	43.7	3.83	
Compound I Day 1-3--Comp. I + E^2 0.1 µg (s.c.) Day 4	843	1000 µg	6	40.8	2.41	44.0
	845	"	6	40.6	1.87	
	906	"	6	50.6	2.63	
Compound I Day 1-3--Comp. I + E^2 0.1 µg (s.c.) Day 4-6	843	1000 µg	6	41.7	1,92	41,8
	845	"	6	43.4	1.41	
	906	"	6	40.2	1.49	

Table XII (cont'd)

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Compound I Day 1-3--Comp. I + E^2 0.1 µg (s.c.) Day 4-8	843	1000 µg	6	37.1	2.07	40.1
	845	"	6	39.9	2.88	
	906	"	6	40.2	0.95	
	934	"	6	43.2	2.28	
Compound I Day 1-3--E^2 0.1 µg (s.c.) Day 4	822	1000 µg	6	32.9	1.50	35.6
	847	"	6	38.2	1.95	
Compound I Day 1-3--E^2 0.1 µg (s.c.) Day 4-6	822	1000 µg	6	35.2	2.50	38.2
	847	"	6	41.1	1.72	
Compound I Day 1-3--E^2 0.1 µg (s.c.) Day 4-8	822	1000 µg	6	36.7	3.05	44.8
	847	"	6	52.9	2.75	
Compound I Day 1-3--E^2 0.1 µg (s.c.) Day 4-13	822	1000 µg	6	44.3	3.01	44.3
Compound I Day 1-3--Comp. I + Tamoxifen 1000 µg Day 4	843	1000 µg	5	47.0	2.56	50.6
	845	"	6	53.6	2.64	
	906	"	6	51.3	2.98	
Compound I Day 1-3--Comp. I + Tamoxifen 1000 µg Day 4-6	843	1000 µg	6	55.7	5.95	57.0
	845	"	6	60.5	3.62	
	906	"	6	54.9	3.84	
Compound I Day 1-3--Comp. I + Tamoxifen 1000 µg Day 4-8	843	1000 µg	6	55.5	4.62	61.9
	854	"	6	56.3	1.86	
	906	"	6	73.8	5.43	

The results of these tests show that compound I is more effective in blocking the uterotropic effect of later-administered uterotropic agents than is compound II. Following pretreatment with compound I, further uterotropic response from estradiol treatment was completely blocked. Estradiol caused some uterine hypertrophy in animals treated similarly with compound II. The

animals treated only with estradiol, after the initial compound treatments, illustrate the difference in duration of action of the compounds. Those treated with compound II exhibit marked hypertrophy between 5 and 10 days of estradiol treatment, while the animals treated with compound I show no such effect.

When animals pre-treated with compound II were then treated with tamoxifen plus compound II, a degree of uterine hypertrophy was observed which I recognize from prior experience as approximating the full uterotropic potential of tamoxifen, in immature rats. In contrast, treatment with tamoxifen plus compound I partially blocked the effect of tamoxifen.

Naphthalene Antiestrogens

An earlier series of antiestrogenic drugs were the naphthalenes, having a basic side chain identical to the amino-ethoxybenzoyl portion of compounds I and II. Data comparing piperidino and pyrrolidino members of the naphthalene series will be presented to assure that the record is complete. The compounds to be tested are as follows:

A. 2-(4-methoxyphenyl)-1-[4-(2-piperidinoethoxy)benzoyl]-3,4-dihydronaphthalene, methanesulfonate

B. 2-(4-methoxyphenyl)-1-(4-(2-piperidinoethoxy)benzoyl]-3,4-dihydronaphthalene, citrate

C. 2-(4-methoxyphenyl)-1-[4-(2-pyrrolidinoethoxy)benzoyl]-3,4-dihydronaphthalene, methanesulfonate

D. 2-(4-methoxyphenyl)-1-[4-(2-pyrrolidinoethoxy)benzoyl]-3,4-dihydronaphthalene, citrate

Uterotropic and Antiuterotropic Tests

The uterotropic and antiuterotropic test methods described in the specification of the above-named patent application were used to evaluate the effects of compounds A-D in immature mice.

In these tests, the standard uterotropic agent was estrone, rather than estradiol. The doses and the sizes of the groups of animals used in the various experiments are explained in Table 13 below. Both subcutaneous and oral administration of the test compounds were used in various assays.

Table XIII

UTEROTROPIC ACTIVITY OF SUBCUTANEOUSLY ADMINISTERED NAPHTHALENES IN IMMATURE MICE

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	719			20.5	1.5	17.9
	725			17.8	0.92	
	728			15.4	1.0	
Compound A	725	100 µg	10	30.7	1.03	30.7
Compound C	719	100 µg	10	39.6	1.0	39.6
Control	710		10	7.6	0.54	9.4
	713		10	11.1	0.83	
Compound B	710	100 µg	10	20.1	1.36	20.4
	713	"	10	20.7	0.39	
	713	33 µg	10	24.1	1.19	24.1
	713	10 µg	10	24.5	1.00	24.5
	713	3.3µg	10	25.1	0.80	25.1
Control	677		10	9.0	0.4	10.1
	713		10	11.1	0.83	
Compound D	677	100 µg	10	37.4	3.0	36.1
	713	"	10	34.8	1.3	
	713	33 µg	10	33.0	1.4	33.0
	713	10 µg	10	33.5	1.5	33.5
	713	3.3µg	10	43.6	2.6	43.6

Table XIV

ANTIUTEROTROPIC ACTIVITY OF SUBCUTANEOUSLY ADMINISTERED NAPHTHALENES IN IMMATURE MICE

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Control	718		10	13.8	0.9	14.6
	729		10	15.4	1.0	

Table XIV (cont'd)

	Assay	Dose of Compound Per Day	N	Mean Uterine Weight (mg)	Standard Error	Mean of Means
Estrone 0.1 µg (s.c.)	718		10	63.6	2.4	59.5
	729		10	55.3	3.4	
Compound A	718	100 µg	10	33.3	2.76	33.3
	718	33 µg	10	36.4	1.70	36.4
	718	10 µg	10	36.5	2.49	36.5
	718	3.3µg	10	53.2	4.16	53.2
Compound C	718	100 µg	10	41.6	2.6	41.6
	718	33 µg	10	36.9	1.6	36.8
	729	"	10	36.6	2.6	
	718	10 µg	10	50.7	3.4	47.2
	729	"	10	43.6	2.9	
	718	3.3µg	10	52.2	2.4	47.6
	729	"	10	42.9	3.0	
Control	711		10	7.6	0.54	9.6
	712		12	10.1	0.76	
	714		10	11.1	0.83	
Estrone	711	0.1µg	10	48.2	3.75	50.8
	712	"	10	51.7	2.63	
	714	"	10	52.6	2.45	
Estrone 0.1 µg (s.c.) + Compound B	711	100 µg	9	22.0	0.73	22.0
	714	10 µg	10	27.3	1.15	27.3
Estrone 0.1 µg (s.c.) + Compound B (oral)	712	100 µg	10	22.6	1.26	22.6
	712	33 µg	10	23.3	0.98	23.3
	712	10 µg	10	26.0	1.04	26.0
Control	678		10	9.0	0.4	10.4
	690		10	12.0	0.6	
	712B		10	10.1	0.9	
Estrone 0.1 µg (s.c.)	678		10	52.8	2.7	52.6
	690		10	53.4	3.3	
	712B		10	51.6	2.6	
Estrone 0.1 µg (s.c.) + Compound D	678	100 µg	10	33.4	2.2	33.4
	678	10 µg	10	43.7	1.7	43.7
Estrone 0.1 µg (s.c.) + Compound D (oral)	690	100 µg	10	28.8	1.7	29.3
	712B	"	10	29.8	1.4	
	690	30 µg	10	32.9	0.8	32.8
	712B	"	10	32.6	1.2	
	690	10 µg	10	36.8	1.5	37.7
	712B	10 µg	10	38.6	2.0	

I conclude from my study of the results of the above experiments that compounds A and B, the piperidino naphthalenes, are more antiuterotropic and less uterotropic than are compounds C and D, the pyrrolidino naphthalenes.

Relative Binding Affinity Tests

Compound A, B, C, and D were tested by the same relative binding affinity method described in the above-named patent application to compare their ability to bind to cytosol estrogen receptor obtained from immature rat uteri. The tests were carried out only at 4°C. In these tests, as in the other relative binding affinity tests, the standard is estradiol, the result for which is 1.0 by definition.

Table XV

RELATIVE BINDING AFFINITY OF NAPHTHALENES FOR IMMATURE RAT UTERINE CYTOSOL ESTROGEN RECEPTORS AT 4°C

	Assay	Relative Binding Affinity (Estradiol = 1.0)
Compound A	1183	.71
	1189	1.00
	1190	2.30
	1192	1.58
	1196	.46
	1227	3.09
Mean		1.52
Standard Error		0.41
Compound C	1189	1.79
	1192	3.45
	1196	1.82
	1198	1.00
	1227	7.14
	1271	2.08
	1275	1.28
Mean		2.65
Standard Error		0.80

Table XV (cont'd)

	Assay	Relative Binding Affinity (Estradiol = 1.0
Compound D	985	.45
	992	.77
	1000	.24
	1183	.50
	1271	.18
Mean		.43
Standard Error		0.10
Compound B	1161	.41
	1183	2.00
Mean		1.20

The results of the relative binding affinity tests are not conclusive. The methanesulfonates, compounds A and C, indicate that both compounds are quite effective, and that the pyrrolidine, compound C, is more effective than is the piperidine, but the tests of the citrate salts indicate that neither is very effective, but that the piperidine is more effective than the pyrrolidine. Thus, no conclusion can be drawn from the relative binding affinity tests of these compounds.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Larry J. Black

Larry J. Black

11/12/82

Date